JC05 Rec'd PCT/PTO 11 JUL 2001

FORM PTO-1390 (REV. 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER 60034-301801

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/889327

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/KR00/00026	14 January 2000 (14.01.00)	14 January 1999 (14.01.99)

TITLE OF INVENTION
Recombinant Enzyme With Excellent D-Amino Acid Oxidase Activity and Production Thereof

APPLICANT(S) FOR DO/EO/US Kang, Yong Ho.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:
    Postcard
    Check Number 126146 in the amount of $1,000.00

JC18 Rec'd PCT/PTO 11 JUL 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/889327	PCT/KR00/00026	60034-301801

21. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

	CALCULATIONS	PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,000	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$
Total claims	5 - 20 =	0	x **$18.00**	$
Independent claims	3 - 3 =	0	x **$80.00**	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$

TOTAL OF ABOVE CALCULATIONS =	$ 1,000	
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +	$	
SUBTOTAL =	$ 1,000	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$ 1,000	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$	
TOTAL FEES ENCLOSED =	$ 1,000	
	Amount to be refunded:	$
	charged:	$

a. [X] A check in the amount of $ 1,000 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. ________ in the amount of $ ________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-3964. A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Paul L. Hickman
Oppenheimer Wolff & Donnelly LLP
P.O. Box 52037
Palo Alto, CA 94303-0746

SIGNATURE

Paul L. Hickman
NAME

28,516
REGISTRATION NUMBER

RECOMBINANT ENZYME WITH EXCELLENT D-AMINO ACID OXIDASE ACTIVITY AND PRODUCTION THEREOF

TECHNICAL FIELD

The present invention relates to a recombinant enzyme with an improved D-amino acid oxidase activity. More particularly, the present invention relates to a D-amino acid oxidase which is fused with a bacterial hemoglobin and shows an excellent efficiency in converting cephalosporin C into glutaryl-7-aminocephalosporanic acid (glutaryl-7ACA) in a bioreactor. Also, the present invention is concerned with a method for producing such a recombinant enzyme.

BACKGROUND ART

With a share of as much as 40 % in the world market, semi-synthetic cephalosporin antibiotics are safer than other antibiotics and have antibacterial activity over a broad spectrum of bacteria. Usually, the chemical synthesis of semi-synthetic cephalosporin antibiotics is started from 7-aminocephalosporanic acid (7-ACA) which is conventionally prepared by chemically cleaving the aminoadipyl residue at position 7 in cephalosporin C that is purified from a microbial product.

The chemical processes including the cleavage of the aminoadipyl residue at position 7 inevitably produce pollution of the environment on account of toxic chemical reagents used and require a tremendous quantity of energy due to their ultra-low temperature reactions. In addition, there is international tendency toward the severe restriction of the organic solvent remaining in the final product. In result, there remains a need for developing processes which can substitute the chemical processes without producing pollution of the environment and allowing the toxic solvents to remain in the final product.

In this regard, bioprocesses have attracted intense attention. Particularly in preparing 7-aminocephalosporanic acid, advantage has been taken of enzymes of microbes. Such bioprocesses using enzymes of microbes are usually conducted in aqueous solution at room temperature and thus. require special

facilities in aspects of energy management and waste water treatment, enjoying the advantage of greatly reducing the production cost of 7-aminocephalosporanic acid.

Microbial conversion of cephalosporin C into 7-aminocephalosporanic acid is conducted in two enzymatic steps: cephalosporin C is oxidated into glutaryl-7ACA by D-amino acid oxidase and glutaryl-7ACA is cleaved at the bond between the glutaryl moiety and the 7-ACA moiety by glutaryl-7ACA acylase.

The D-amino acid oxidases obtained from eucaryotes including *Trigonopsis variabilis*, *Rhodotorula gracilis*, *Rhodotorula glutinis* and *Fusarium solani* have been used for the microbial conversion of cephalosporin C, thus far. The D-amino acid oxidases of such eucaryotes use FAD as a coenzyme. Thus, during their catalytic oxidation of cephalosporin C, oxygen atoms are always required as an electron acceptor. Since oxygen has extremely low solubility in water, a sufficiently large amount of oxygen must be continuously supplied to the bioreactor in order to achieve performance of the D-amino acid oxidase.

Most enzyme bioreactors employ matrixes in which enzymes are immobilized for reuse. When the D amino oxidases are immobilized in matrixes, however, very poor conversion yields of cephalosporin C are obtained because oxygen molecules cannot be readily diffused in the matrixes. In order to overcome this problem, the oxygen partial pressure in the bioreactor is raised. However, the oxygen pressure increase forces the bioreactor to be specially constructed in addition to being economically unfavorable owing to loss of a large quantity of oxygen.

DISCLOSURE OF THE INVENTION

Leading to the present invention, the intensive and thorough research on the bioconversion of cephalosporin C, repeated by the present inventor aiming to efficiently provide oxygen for immobilized D-amino acid oxidase resulted in the finding that, when an oxygen-carrying molecule was immobilized together with D-amino acid oxidase, the catalysis of the enzyme could be performed without a shortage of oxygen supply and that bacterial hemoglobin was effective as the oxygen-carrying molecule.

Therefore, it is an object of the present invention to provide a recombinant enzyme which shows stable and excellent amino acid oxidase activity when being

applied to a bioreactor for converting cephalosporin C into 7-aminocephalosporanic acid.

It is another object of the present invention to provide a method for producing such a recombinant enzyme.

Based on the present invention, the above objects could be accomplished by fusing a bacterial hemoglobin (*Vitroscilla* hemoglobin) gene and a D-amino acid oxidase gene to each other by a polymerase chain reaction to give a fusion gene, inserting the fusion gene in an expression vector, expressing the fusion gene in *E. coli*, purifying the fusion enzyme, and immobilizing the fusion enzyme in a polyacrylamide matrix to convert cephalosporin C.

BEST MODES FOR CARRYING OUT THE INVENTION

In the present invention, a bacterial hemoglobin gene, for example, *Vitreoscilla* hemoglobin (hereinafter referred to as "VHb") gene, is fused to a D-amino acid oxidase (hereinafter referred to as "D-AAO") by PCR. In this regard, a stretch of DNA in a 5' end region of the VHb gene is designed as a sense primer while a stretch of DNA in a 3' end region of the VHb gene is used as an antisense primer which has an overlapped portion with a stretch of DNA in a 5' end region of the D-AAO gene. Likewise, a sense primer for the amplification of the D-AAO gene is designed to have an overlapped portion with a stretch of DNA in 3' end region of the VHb gene. With respective primer sets, the VHb gene and the D-AAO gene are amplified. For fusion, these PCR products are mixed and re-amplified by use of a primer set consisting of the sense primer used to amplify the VHb gene and the antisense primer used to amplify the D-AAO gene. Alternatively, the VHb gene and D-AAO gene are mixed and may be fused by PCR in a DNA shuffling fashion without using primers.

Next, the VHb-DAAO fusion gene is introduced into an expression vector and expressed.

The catalytic activity of the recombinant enzyme can be measured by detecting the amount of H_2O_2, which is side-produced during the conversion of cephalosporin C into 7-aminocephalosporanic acid, in luminometric analysis.

To proceed with the research of the present invention, vector pUC8:16 carrying a VHb gene was granted from Professor Benjamin C. Stark, Illinois

Institute of Technology. After being deprived of its self promoter, the vector was amplified at the VHb gene region with reference to the reported gene sequence (Khosla and Bailey, 1988, Mol. Gen. Genet, 214:158-161; Dikshit and Webster, 1988: Gene 70:377-386).

As for the D-AAA gene used in the present invention, it was derived from *Trigonopsis variabilis* or *Rhodotorula gracilis*. These microogranisms were obtained from American Type Culture Collection: *Trigonopsis variabilis* ATCC10679 and *Rhodotorula gracilis* ATCC26217. From each of these microbes, genomic DNA was isolated, and used as a substrate to amplify a D-AAO gene (cDNA). For the cloning and the expressing of the D-AAO gene, commercially available vectors pALTER-EX2 (Promega, USA) and pKK223-3 (Pharmacia Biotech, Sweden) were utilized. PCR mixtures for the amplification of the genes of interest are given in Table 1, below.

TABLE 1

PCR Mixture Composition

	DNA	25mM $MgCl_2$	10X Buffer	DH_2O	2.5Mm dNTP	Taq polymerase	Primer
VHb	1μl	4μl	10μl	79μl	1μl	5 units	200pM
T. variabilis	2μl	4μl	10μl	79μl	1μl	5 units	200pM
R. gracilis	2μl	4μl	10μl	79μl	1μl	5 units	200pM

PCR was carried out in a thermal cycler, such as that manufactured by EquiBio. Belgium, identified as "ThermoJet", with 35 cycles of denaturing temperature at 94 °C for 1 min, annealing temperature at 55 °C for 1 min and extending temperature at 72 °C for 1 min, finally followed by 74 °C extension for an additional 4 min.

Taking advantage of the H_2O_2 side-produced during the bio-conversion of cephalosporin C, luminometry for analyzing the activity of the D-AAO used in the present invention is based on the following chemical reaction formulas:

D-AAO+Cephalosporin $C+H_2O_2+O_2 \rightarrow$ AKA-7ACA$+NH_3+H_2O_2$

$$H_2O_2+2OH^-+\text{Luminol}+\text{Peroxidase}\rightarrow\text{4-Aminophthalate}+N_2+2H_2O+\text{Light}$$

This analytic method can determine the activity of the recombinant enzyme of the present invention very rapidly and accurately.

For the analysis of the recombinant enzyme, the recombinant vector of the present invention is introduced into *E. coli* which is, then, cultured in an LB broth. The cultured cells are harvested by centrifugation, washed with phosphate buffered saline (PBS, pH 7), and added with a solution containing cephalosporin C 20 mM, luminol 2 mM, peroxidase 1 unit, and FAD 5 μM. Using a luminometer (Tuner design, USA), the quantity of light emitted for 20 sec is measured. From this, the quantity of H_2O_2 is determined by use of a standard curve.

EXAMPLE 1

Fusion of VHb Gene and D-AAO Gene By PCR

In order to amplify a VHb gene, a stretch of DNA in a 5' end region of the VHb gene was designed as a sense primer while a stretch of DNA in a 3' end region of the VHb gene was used as an antisense primer which was so designed as to have an overlapped portion with a stretch of DNA in a 5' end region of the D-AAO gene. Likewise, a sense primer for the amplification of the D-AAO gene was designed to have an overlapped portion with a stretch of DNA in 3' end region of the VHb gene.

The DNA fragments thus amplified were purified and mixed with each other. In combination with the sense primer used to amplify the VHb gene and the antisense primer used to amplify the D-AAO gene, the amplified gene mixture was subjected to PCR with 35 cycles of denaturing temperature at 94 °C for 1 min, annealing temperature at 55 °C for 1 min and extending temperature at 72 °C for 1 min, finally followed by 74 °C extension for additional 4 min. The PCR composition used in this fusion PCR is given in Table 2, below.

TABLE 2

PCR Mixture Composition For VHb-DAAO Gene Fusion

	DNA	$MgCl_2$ (25mM)	10X buffer	dH_2O	dNTP (2.5mM)	Taq Polymerase	Primer
VHb	1μl	4μl	10μl	78μl	1μl	5 units	200pM
D-AAO	1μl	4μl	10μl	78μl	1μl	5 units	200pM

EXAMPLE 2

Fusion of VHb gene and D-AAO gene By DNA Shuffling

The VHb and the D-AAO DNA fragments amplified in Example 1 were purified and mixed with each other. The mixture was subjected to PCR without primers. The PCR is carried out with 35 cycles of denaturing temperature at 94 °C for 1 min, annealing temperature at 55 °C for 1 min and extending temperature at 72 °C for 1 min, finally followed by 74 °C extension for additional 4 min. The PCR composition in this fusion PCR is given in Table 3, below.

TABLE 3

PCR Mixture Composition For VHb-DAAO Gene Fusion

	DNA	$MgCl_2$	10X Buffer	dH_2O	DNTP (2.5mM)	Taq polymerase
VHb	10μl	4μl	10μl	64μl	1μl	5 units
D-AAO	10μl	4μl	10μl	64μl	1μl	5 units

EXAMPLE 3

Cloning of VHb-DAAO Fusion Gene

To produce blunt ends, VHb-DAAO fusion DNA fragments amplified in Examples 1 and 2 were treated with Klenow enzyme at 25 °C for 30 min in a Klenow mixture containing a Klenow fragment 4 units, dNTP (2.5 mM) 3 μl, and

10x buffer 3 μl. The blunt-ended fusion DNA fragments were purified by ethanol precipitation, and sub-cloned in expression vectors, respectively.

For the sub-cloning, pALTER-Ex2 and pKK223-3 were linearlized at *Stu*I and *Sma*I, respectively, and dephosphorylated with alkaline phosphatase, followed by incubation for 1 hour at 16 °C along with the fusion gene fragment and T4 DNA ligase.

EXAMPLE 4

Bio-Conversion of Cephalosporin C in Packed Bed Bioreactor

E. coli was transformed with the vectors constructed in Example 3, and cultured overnight in LB broth to obtain cell extracts. These cell extracts were precipitated by ammonium sulfate and subjected to dialysis, followed by passing the dialysates through anionic exchange resins (DEAE-Sephadex FF) to purify D-AAO and VHb-DAAO, respectively. These purified enzymes were immobilized in polyacrylamide matrixes which were then cut into cubes (1.5 x 1.5 x 1.5 mm) and put in packed bed bioreactors (1.5 cm in diameter, 15 cm in length).

20 mM cephalosporin C in Tris-HCl buffer (pH 8) was circulated at a flow rate of 1.5 mL/min through the packed bed bioreactors with the aid of a peristaltic pump while oxygen was continuously supplied to the batch type vessels. At regular time intervals, samples were taken from the reactors and quantitatively measured for the H_2O_2 produced as a result of the bioconversion of cephalosporin C. The results are given in Table 4, below. As indicated in Table 4, the by-product H_2O_2 was hardly produced in the D-AAO immobilized reactor because of the oxygen deficiency resulting from the resistance of the matrix to oxygen diffusion while the VHb-DAAO fusion enzyme immobilized reactor allowed H_2O_2 to be produced at an amount 12 times as much as that of the D-AAO immobilized reactor within 45 min. Therefore, the VHb-DAAO fusion enzyme of the present invention could effectively perform the conversion of cephalosporin C without increasing the oxygen partial pressure in the reactor.

The novel recombinant *E. coli*, which was transformed with the recombinant vector pALTER-Ex2 carrying the VHb-DAAO fusion gene of the present invention, was deposited in the Korean Collection for Type Culture at

Korea Research Institute of Bioscience and Biotechnology (KRIBB) under the deposition No. KCTC 8923P on Jan. 18, 1999.

TABLE 4

Conversion Ability of Recombinant D-Amino Acid Oxidases in Terms of Production of H_2O_2

Time Period (min)		0	15	30	45	60	90	120
H_2O_2(μM)	D-AAO	0	0.5	0.5	0.8	1.0	1.0	1.0
	VHb-DAAO	0	2.0	4.5	12.0	12.0	12.0	12.0

INDUSTRIAL APPLICABILITY

As elucidated in the above examples, the recombinant enzyme VHb-DAAO can be obtained from the novel recombinant E. coli, which harbors a fusion gene consisting of a *Vitreoscilla* hemoglobin gene and a D-amino acid oxidase and can be applied to a bioreactor which can industrially convert cephalosporin C into glutaryl-7ACA.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM AND OTHER BIOLOGICAL MATERIALS

A. The indications made below relate to the deposited microorganism and other biological materials referred to in the description on Page 9, Lines 14 – 16	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution (*including postal code and country*): The Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology #52, Oun-Dong, Yusong-Gu, Taejon, 305-333, Korea	
Date of deposit January 18, 1999	Accession Number KCTC 8923P
C. ADDITIONAL INDICATIONS(*leave blank if not applicable*): This information is continued on an additional sheet ☐	
D. CHARACTERISTICS FOR WHICH INDICATIONS ARE MADE	
E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)	
The indications listed below will be submitted to the International Bureau later	

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on
Authorized officer	Authorized officer

CLAIMS

1. A recombinant fusion enzyme VHb-DAAO, expressible from a recombinant fusion gene consisting of a gene encoding a bacterial hemoglobin and a gene encoding a D-amino acid oxidase.

2. A recombinant fusion enzyme bacterial hemoglobin-D-amino acid oxidase as set forth in claim 1, wherein said bacterial hemoglobin contains a full or a partial length of a *Vitreoscilla* hemoglobin peptide sequence or its functionally analogous peptide sequence.

3. A method for producing a recombinant fusion enzyme VHb-DAAO, which comprises fusing a bacterial hemoglobin gene and a D-amino acid oxidase gene to each other by a polymerase chain reaction to give a fusion gene, inserting the fusion gene in an expression vector, expressing the fusion gene in *E. coli*, and purifying the fused enzyme VHb-DAAO.

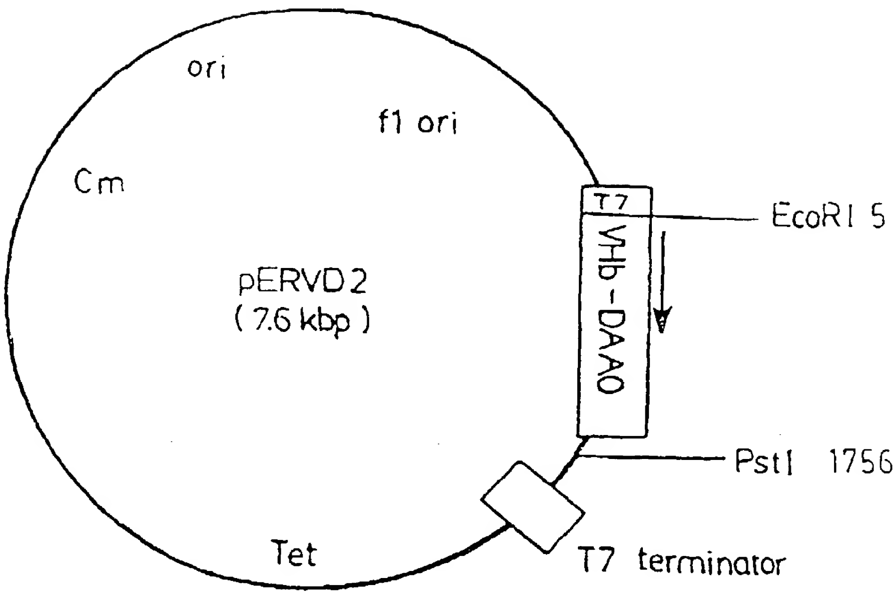
4. A recombinant vector pALTER-EX2/VHb-DAAO, which is constructed by introducing a fusion gene consisting of a bacterial hemoglobin gene and a D-amino acid oxidase.

5. A recombinant E. coli (KCTC 8923P), which is transformed with the recombinant vector pALTER-EX2/VHb-DAAO of claim 4.

ABSTRACT

Disclosed is a recombinant enzyme which can convert cephalosprin C into glutaryl-7-aminocephalosprin acid in a bioreactor at a high yield. A bacterial hemoglobin (*Vitreoscilla* hemoglobin) gene and a D—amino acid oxidase gene are fused to each other by PCR and the fused DNA fragment is cloned and express in *E.coli*. In a bioreactor, the recombiant enzyme VHb-DAAO can sufficiently supply oxygen as an electron acceptor by virtue of the fused hemoglobin, thereby showing an excellent capability of converting cephalosporin C.

FIG. 1



Cm: Coding site of Choloramphenicol interfered gene

Tet: Coding site of Tetracycline interfered gene

T7: T7 RNA Polymerase promoter

O I P E JC26
JUL 3 1 2002
PATENT & TRADEMARK OFFICE

Docket No. 60034-301801

DECLARATION, POWER OF ATTORNEY AND PETITION

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AUG 0 1 2002
OFFICE OF PETITIONS

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"RECOMBINANT ENZYME WITH EXCELLENT D-AMINO ACID OXIDASE ACTIVITY AND PRODUCTION THEREOF"** the specification of which

☐ is attached hereto
☒ was filed on July 11, 2001 as Application Serial No. 09/889,327
and was amended on ________ *(if applicable)*.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) or U.S. provisional application(s) for patent or inventor's certificate listed below and have also identified below any foreign application or U.S. provisional application(s) for patent or inventor's certificate having a filing date before that of the application of which priority is claimed.

Prior Foreign/U.S. Provisional Application(s)

(Number)	(Country)	(Day, month, year filed)	Priority Claimed Yes	Priority Claimed No
No. 1999-865	Korea	January 14, 1999	☐	☒
No. 1999-5580	Korea	February 19, 1999	☐	☒
			☐	☐

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	Filing Date	(Status: Patented, pending, abandoned)
PCT/KR00/00026	14 January 2000 (14.01.00)	Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint G.P. SMITH, REG. 20,142; A.C. ROSE, REG. 17,047; L.J. BOVASSO, REG. 24,075; C. BERMAN, REG. 29,249; C. DARROW, REG. 30,166; M.E. HARRIS, REG. 26,690; K.A. MACLEAN, REG. 31,118; C. ROSENBERG, REG. 31,464; M.E. BROWN, REG. 28,590; S.R. HANSEN, REG. 38,486; D.N. LARSON, REG. 29,401; J.W. INSKEEP, REG.

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Docket No. 60034-301801

33,910; H.D. JASTRAM, REG. 19,777; B. CANTER, REG. 34,792; C.J. LERVICK, REG. 35,244; L. CULLMAN, REG. 39,645; C.A.S. HAMRICK, REG. 22,586; R.O. GUILLOT, REG. 28,852; J. BOYCE, REG. 40,920; C. CHOU, REG. 41,672; A.B. DIEPENBROCK III, REG. 39,960; M.K. BOSWORTH, REG. 28,186, L. SHERRY, REG. 43,918; L. McROSS, REG. 40,427; T. KHAN, REG. 46,273; L. GUERNSEY REG. 40,008; M. HUGHES, REG. 29,077; R. ROBERTS, REG. 38,597; S. HOWELL, REG. 45,929; R. NADER, P47,260; B. COLEMAN, REG. 39,145; P. HICKMAN, REG. 28,516; J. KUDLA, REG. P47, 724; D. BURTON, REG. 45,323; S. KELLEY, REG. 43,449; OPPENHEIMER WOLFF & DONNELLY LLP, 1400 Page Mill Road, Palo Alto, California 94304, (650) 320-4000, as my attorneys with full power of substitution and revocation, to prosecute said application and to transact in connection therewith all business in the Patent and Trademark Office and before competent International Authorities.

COPY

Address all telephone calls to Paul Hickman at (650) 320-4000 and address all correspondence to:

Paul Hickman, Esq.
OPPENHEIMER WOLFF & DONNELLY LLP
P.O. Box 52037
Palo Alto, California 94303-0746

Wherefore I pray that Letters Patent be granted to me for the invention or discovery described and claimed in the foregoing specification and claims, and I hereby subscribe my name to the foregoing specification and claims, declaration, power of attorney, and this petition.

Full Name of Sole or First Inventor:	Kang, Yong Ho
Home Address:	Yongnam University, Applied Microbiology Dept., 712-749 Dae-dong, Kyongsan-city, Kyongsangbuk-do, Republic of Korea
Post Office Address:	Same as above
Citizenship:	Republic of Korea
Inventor's Signature:	Kang Yong Ho Date: Sep. 4, 2001

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1751

PATENT

530 Rec'd PCT/PTO 25 JUN 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OIPE JC184 JUN 25 2002 PATENT & TRADEMARK OFFICE

In re application of: KANG, YONG HO

Serial No.: 09/889,327

Filed: 7/11/2001

GAU/Examiner: 1751/UNASSIGNED

For: RECOMBINANT ENZYME WITH EXCELLENT D-AMINO OXIDASE ACTIVITY A ND PRODUCTION THEREOF

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Signed: Maria Castellano

CHANGE OF ATTORNEY'S ADDRESS IN APPLICATION

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Commissioner for Patents
Washington, D. C. 20231

Sir:

Please send all correspondence for this application as follows:

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Dated: 6/18/02

Respectfully submitted,

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